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THE OÖGENESIS AND EARLY DEVELOPMENT OF HYDRA.[1]

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During the winter of 1904–05 the writer took up the problem of the oögenesis of *Hydra*, especial attention being given to the cytological changes involved in the development of the egg. The species upon which the work was done was judged to be *H. grisea.* The animals were found growing in large numbers in a pond near Syracuse, N. Y. The results obtained were sufficiently marked to warrant publication, but the paper has been withheld until some of the points involved might receive confirmation from new material.

In the meantime there have been published two papers bearing upon the same subject: one by Tannreuther ('08) and another by Downing ('08). In general agreement with the results obtained by these two as well as by previous writers, were my own, but in some important particulars they differ widely. For several months past an abundance of sexually reproducing *Hydra* have been kept growing in the laboratory, and material taken therefrom has afforded ready confirmation of the results previously obtained. These results have been reviewed, and, considerably abbreviated, are here presented essentially unchanged. Some discussion of points suggested in recent papers has been inserted, and those particulars in which agreement with other workers exists have been but slightly dwelt upon.

As above stated, *Hydras* have been kept growing in the laboratory. Observations on these animals yielded interesting results. Early in October, 1907, collections were made from a small stream near Potsdam, N. Y., and the animals brought

[1] Contributions from the Zoölogical Laboratory, Syracuse University.

into the laboratory were placed in large gallon aquaria jars. In the jars were several species of unicellular, and some more highly organized plants in sufficient quantity to oxygenate the water. A few small pond snails were valuable in keeping the jars clean. In about two or three weeks after collection the animals began to bud very actively, and the dozen or so in each jar soon increased greatly in numbers. In one jar it was estimated that there were between two and three hundred individuals. This asexual period lasted between ten days and two weeks when there began an active sexual phase apparently as marked as had been the asexual. Throughout the winter months, and indeed, until observations were discontinued in July, 1908, this alternation of asexual and sexual periods was constant. At no time, however, were the reproductive activities entirely of one sort, but that there were periods of greatest sexual and of asexual development there could be no doubt. The same cultures have been kept under observation during the past year and somewhat similar though less marked conditions have been noted. The long vacation period during which they were without attention brought them to the fall of 1908 in a somewhat depleted condition. With fresh (distilled) water and food they began again to bud and produce eggs and spermaries. Their activities during the past few months, however, have been much less marked than formerly. This, I believe, to have been due in large part to the accumulation in the water of metabolic products deleterious to the normal activities of the animal. When taken from the jars and placed in fresh water with a plentiful supply of food, they began at once to bud rapidly and after a time to produce both eggs and spermaries, though, it must be said, with a vigor less than when the culture was first started. In proper environment, then, the *Hydra* will for a long time continue to reproduce both asexually and sexually, and the maximum development of each phase occurs in alternating cycles, or periods.

The embryos developed during the first sexual period were entirely of the flattened, short-spiculated form figured by Korotneff ('83) and Brauer ('91*a*) as that of *H. fusca.* These same animals, or at least their descendants, thereafter produced only the spherical heavily spiculate embryos figured by Kleinenberg

('72) as that of *H. aurantiaca*, and by Brauer (*op. cit.*) as belonging to *H. grisea*. It seems hardly possible that animals of both species (assuming *H. aurantiaca* and *H. grisea* to be identical) could have been taken under the same conditions and developed to such numbers at the same time. If but one species was present then it produced both forms of the embryo, while if both were present then one must have entirely disappeared and given place to the other. The latter alternative must have been attended by a marked diminution in the number of the animals immediately following the first sexual period, and no such observation was made. It appears then that the character of the embryo cannot be taken as a constant and specific difference. Brauer ('91*a*) uses this characteristic, together with the manner in which the embryo leaves the body of the mother, in separating *H. grisea* and *H. fusca*:

"*Hydra grisea* L.: Ei fallt ab, Form Kuglig, Schale ringsum mit grossen, an der Spitze meistverzweigten Zacken besetzt."

"*Hydra fusca* L.: Eier werden einzeln angeklebt, Form unten flach, oben konvex, Schale nur auf der oberen Seite mit Kurzen Stacheln besetzt."

An explanation of the cause for this unusual embryo-formation is not at hand. The conditions under which the spiculate embryos were produced were, so far as one could observe, the same as those under which the flattened, short-spiculate type were formed. It is rather remarkable that during the eighteen months and more that the animals were kept under observation, only the spherical, heavily spiculate embryos were to be found after the first sexual period during which the alternate type was produced. Moreover, the color of the animals under different conditions varied greatly, as did also their size and disposition toward sexual or non-sexual reproduction. Experiments were performed to determine, if possible, the relation of color, size, and reproductive proclivities to environmental factors. These were not altogether conclusive, but will be presented in another paper. In general, however, one may say that so widely do the animals vary under different conditions that one might easily mistake for a different species the forms encountered at either extremity of the varying limit. This, it seems, will readily account for the numerous

species described by the earlier investigators, who, naturally, placed much emphasis upon color and size as specific differences.

As killing agents, hot corrosive-acetic proved very satisfactory when the preparations were to be stained with Heidenhain's iron-hematoxylin. Corrosive sublimate, used both hot and cold, also gave good results. Bouin's mixture proved especially valuable in the study of the pseudo-cell formation.

Origin of the Ovary.

Most investigators of the problem of the oögenesis of *Hydra* have attributed the origin of the ovary to the interstitial cells. My own observations have agreed in this respect. Longitudinal sections show that in the region in which the egg takes its origin there are numerous nests of these minute cells, but in the lower, or aboral end, they are much less numerous, and almost entirely absent near the foot. They are often found dividing mitotically, when there are usually three or four divisions taking place very near together. They vary somewhat in size in different sections, but their appearance and disposition would lead to the following conclusion with regard to their growth.

The very young interstitial cell is possessed of a nucleus which has a very delicate chromatin reticulum with small nodal enlargements. The nucleus, to a great degree, fills the whole cell, there being but a very thin envelop of cytoplasm. With the onset of egg production, the nucleus begins to enlarge and very soon a definite nucleolus can be found, and not infrequently, two are present. The mass of the cytoplasm increases at the same time and as a result of this increase in the size of these cells, a layer of the primative ova, as they have been called, is definitely established. Externally, this is evidenced by the clitellum-like enlargement usually in the upper third of the body. This sometimes completely surrounds the body, or, with perhaps equal frequency, is found on one side only. Cases were noted in which no marked change in the external appearance could be seen, yet the process of egg production took place. The size of the egg is dependent in part upon the quantitative production of these cells, as will become evident from the discussion of their fate. Whether or not

the cells divide after reaching this stage, is rather uncertain. In none of the sections examined were there found conclusive evidences of such divisions taking place either mitotically or amitotically. There were present, however, many cases in which a cell was possessed of two nuclei. It was thought at first that division took place amitotically, for some of the nuclei were markedly elongated, and might easily give foundation for such belief. Such doubly nucleated cells probably arise from the coalescence of two cells.

The two nucleoli, above referred to as frequently being present in the nucleus, are not chemically the same, as they differ in their staining reaction, one staining much more deeply than the other (Fig. 1, Pl. I.). In some cases each of the nucleoli is found to be made up of two parts, in which again, one differs from the other in staining reaction. One appears to be a true nucleolus, while the other probably consists of a droplet of some food element passed through the nuclear membrane. Darkly staining bodies are also found in the meshwork of the cytoplasm (Fig. 1, Pl. I.). These appear to be true metaplasmic bodies, probably food material taken up by the cell. They are not found in all cases, and their presence is probably dependent upon the physiological activities of the cell. Frequently, the nucleolus migrates out of the nucleus by rupturing the nuclear membrane (Fig. 6, Pl. I.). The nucleolus is usually surrounded by a clear non-staining area. The chromatin material forms a fine network often somewhat peripherally arranged. In especially well-stained preparations one can distinguish very delicate threads which apparently hold the nucleolus in position. The nuclei themselves are either round or ellipsoidal in section. In the former case but one nucleolus is present, in the latter, two (Fig. 1, Pl. I.). Doflein ('97) describes similar conditions in the primitive ova of *Tubularia mesembryanthemum*, and Allen ('00) for *T. crocea*.

In the nests of interstitial cells from which the primitive ova are derived, one usually finds nematocysts developing in large numbers. In the course of development of the ovarian area these nematocysts either migrate out, or are resorbed. Frequently they are found within the egg itself.

Growth of the Egg.

The germinal area having been formed as above described, the egg begins its growth. Near the center of the mass of primitive ova there are to be found several which are larger than the rest. These cells lie close to the supporting membrane. Downing ('08) is inclined to regard these cells as the true ova, and is of the opinion that they are always present and recognizable among the interstitial cells of the adult animal. He notes that there are some cells which are spherical in outline, and are possessed of a very large nucleus near which there lies a small dark ovoid body. As noted above, the interstitial cells even in an early stage in the formation of the germinal area, differ considerably in size. The ovoid bodies near the nucleus are to be found in many of the interstitial cells lying near the supporting layer, and are doubtless only a metaplasmic body. It is permissible, certainly, to regard these cells as ova, and, as Downing contends, as true ova, but it would seem to be impossible to show that these large cells do not have precisely the same origin as the remaining ones of smaller size over which they have an advantage by virtue of their nearness to the entoderm from which their food supply is derived. All stages, as regards size, are to be found between these large and even the smallest cells. Unless these large cells do have a different origin than do the small ones, or unless they become markedly different in some respect from the small ones, there is nothing to be gained by regarding them as true egg cells and the small ones, therefore, as something entirely different. That these large cells may be present in the ectoderm is doubtless true. That the egg, in the manner to be described, takes its rise always from these and no other later formed large cells, seems to me to be extremely improbable.

The processes concerned with the growth of the egg are interesting. Tannreuther ('08) has already pointed out that the egg mass originates by the coalescence of a number of the primitive ova, the process beginning in the large cells near the center of the ovarian area. Inasmuch as my own observations were carried on in somewhat greater detail, it may be justifiable to outline the process again.

Reference has already been made to the large cells which,

in part, make up the primitive ova. In these cells begins the coalescing process, which is attended by marked cytological changes. The chromatin network retreats further from the nucleolus (Fig. 2, Pl. I., *a*), and finally assembles as a well-defined coarse spireme (Fig. 2, Pl. I., *b*). Coincidently with this process, the nuclear membrane becomes less distinct and even absent in places. Within the nucleolus of these large, and frequently of even smaller cells, two or three minute vacuoles appear, and these may, in rare cases, number seven or eight. As the nucleus enlarges these vacuoles become larger and finally unite to form one which almost entirely fills the nucleolus; the chromatin filament becomes broken up into short strands; the nuclear membrane disappears entirely, and the nucleolus, frequently filled with the single large vacuole, very often migrates out of the chromatin strands (Fig. 5, Pl. I.). Associated with these processes is the disappearance of the cell-walls of those cells concerned in the changes. They become more and more indistinct and finally disappear entirely, or nearly so, as a result of which the substance of the several cells concerned unites to form a single mass (Fig. 4, Pl. I.). The mass formed by the fusion of these cells is the egg, in process of growth. It is interesting to note that there are several metaplasmic bodies in many of these cells as already described, but that now they are even larger and more pronounced.

The disappearance of the cell walls in the manner above described sets free the nucleus or its parts in the cytoplasmic mass of the egg. The coarse spireme of chromatin material becomes broken up into short strands which become dispersed and disappear entirely, though careful search in the cytoplasm of an egg will frequently reveal fragments whose presence therein is easily explained by the above account of the processes which take place (Fig. 9, Pl. II.).

Not all of the nuclei which contribute to the mass of the egg undergo this fragmenting process. Some persist apparently unchanged even for some time but their ultimate fate, save probably in the case of one, is in the formation of the so-called pseudo-cells which is to be described hereafter.

The egg mass now becomes distinctly vesicular, and imbedded

in it are a number of minute bodies apparently identical with those described as occurring in the primitive ova, and set free here by the coalescence of the cells. Some of them, however, are doubtless nucleoli, or at least nucleolar bodies, which, by the same process, have been added to the already rather complex egg mass.

The egg now continues to increase in size by the further appropriation of the cells surrounding it. The cells thus contributing their mass to that of the egg may undergo a regressive change similar to that described for the large ova which unite to form the growing egg. One of the primitive ova, or possibly at this time rightly called nourishing cells, becomes conspicuously larger than its fellows; its nucleus becomes correspondingly large and the regressive changes above described are repeated here. The chromatin assembles in a loosely coiled coarse spireme; the nuclear membrane disappears; the nucleolus becomes very large and frequently filled by a vacuole; the cell wall between this large cell and the egg gradually becomes absorbed and the two unite. Such a process in an intermediate stage is shown in Fig. 10 (Pl. II.) in which the nuclear membrane of the nourishing cell has entirely disappeared on one side and the chromatin filaments are broken up into short pieces. Two metaplasmic bodies are noticeable in the large cell as well as in the egg itself. Their probable origin has been discussed above. Fig. 9 (Pl. II.) represents a stage a little later than that above referred to. Outside of the egg is a large non-nucleated mass two or three times the size of the ordinary cells. Near it and next to the egg is a smaller cell in which these regressive changes are well advanced. The cell-walls between these two bodies is less distinct than in the other cells in the field, suggesting that the two would eventually unite, before union with the egg. Such conditions as this are not at all rare, and suggest that these large cells are developed by the union of two or more of the nourishing cells rather than by the growth of any one to this unusual size. Having reached this stage the large mass then becomes a part of the egg by the disappearance of the wall between the two bodies, thus setting free into the substance of the egg the chromatin strands, nucleoli and nucleolar bodies as

well as contributing considerably to the proportions of the egg by the addition of a considerable mass of cytoplasm.

It is evident from the above description that there is here an unusual case of nuclear degeneration. Processes of nuclear fragmentation have been described as occurring in the eggs of other cœlenterates, but in a correspondingly later stage in their history and with ultimate results of an entirely different nature. Hickson ('93) has noted nuclear fragmentation in the case of *Distichopora violacea;* Allen ('00) in *Tubularia crocea;* Hargitt ('04) in *Eudendrium* and *Pennaria tiarella.* These cases are, however, quite different in that they consist of a simple breaking up of the nuclear elements into small parts without their entire dissolution. Herrick has described nuclear fragmentations in the Crustacea, viz., in *Alpheus minor, A. sauleyi* and *Homarus americanus* in which the nuclei constrict off small buds somewhat after the fashion of growing yeast. The process takes place for only a limited time in the egg-nauplius stage, and in definite regions. These cases differ widely from the one herein described in which the nucleus breaks down entirely and eventually disappears in the cytoplasm of the egg.

Soon after the egg has increased to a size six or eight times that of its original proportions, the nourishing cells are taken up in an entirely different fashion, and in a manner probably designed for supplying the embryo with energy in the later stages of its development. This process results in the formation of the so-called pseudo-cells. In some cases it does not begin until the egg is nearly one half its matured proportions. The beginning of the process is probably dependent upon the presence within the egg of some chemical compound which incites the changes to be described. The production of this substance is probably accomplished at different periods of development in different eggs.

Inasmuch as I have attempted to work out in some detail the cytological changes involved in the formation of the pseudo-cells, it may not be out of place to insert an outline of the opinions held by some previous investigators with reference to their origin.

Historical.

Kleinenberg ('72) first described the development of the pseudo-cells and proposed the name by which they have since been known. He remarked that they had been the source of many misconceptions, especially on the part of Ecker, who regarded them as true embryonal cells with a nucleus and a cell wall, and as arising by divisions of the original egg cell (*l. c.*, p. 39). He himself set about to find eggs in which all stages in their development could be found and came to the following conclusion with regard to their origin :

"Als Anfange erscheinen sehr kleine kuglig umschreibene Verdichtungen im Plasma, die sich von den jungen noch nicht gefarbten Chlorophyllkorpern nur durch etwas starkeres Lichtbrechungsvermogen unterscheiden. Indem sie sich vergrossern, entsteht in ihrem Innern eine Hohle die zuerst genau in der Mitte gelegen ist, mit dem fortschreitenden Wachsthum aber diese centrale Lage verlasst und sich an einer Stelle der Oberflache nahert. Hier erhebt sich darauf, frei in die Hohle hineinragend, auf breiter Basis ein niedriger kleiner Kegel, der spater eine ungefahr linsenformige Gestalt annimmt und endlich zu dem Zapfen auswachst. Da sich die Eiweisskornchen schon fruher ausgeschieden haben, ist hiermit die Entwicklung der Pseudozellen beendigt" (*l. c.*, p. 40).

This description is for the pseudo-cells of the egg of *H. viridis*. He states that a similar structure exists in those of *H. grisea* and *H. aurantiaca*, except that there is no plug or extension into the cell, but in its place a simple thickening of the rim of the cell which sometimes fills more than half its inner part.

Ciamician ('79) describes the formation of structures which he says "have the greatest similarity to the pseudo-cells found by Kleinenberg in the egg of *Hydra*." He, like Kleinenberg, believed that these bodies grow in the plasma of the egg for he states that "Die Pseudozellen erscheinen genau so wie bei Hydra anfangs als ganz kleine Kugelchen (0.001 mm. Durchmesser) und konnen bis zu einem Durchmesser von 0.005 mm. anwachsen." He describes also the vacuolated condition of some of these bodies and states that by the union of these vacuoles a certain nucleus-like part is cut out of the main mass. He found, too, that

these cells frequently divided into two, three, four or even six parts, usually all of unequal size. This process always took place before the cutting out of the central nuclear-like portion.

Brauer ('91*a*, p. 170) makes mention of the "Dotterkernen, der sogenannten Pseudozellen." In describing the growth of the egg (p. 178) he speaks of "die Auflosung der Nahrzellen und ihre Aufnahme als sogenannte Pseudozellen durch die Eizellen." Further on in the same article (p. 184) he remarks that the structure of these bodies has been already described by Kleinenberg, implying that his own observations agreed with those of the last named author. In another paper of the same year ('91*b*) he describes the origin of the pseudo-cells as follows: "Mit dem Wachsthum der Eizelle . . . beginnt die Auflosung der Nahrzellen, indem die Umrisse unregelmassig werden und der Kern verschwindet. Gleichzeitig treten auch die Pseudozellen als kleine, von Anfangs an sich intensiv farbende Kugelchen auf, welche rasch heranwachsen und die eigenthumliche Kernahnliche Form, wie Ciamician und Tichomiroff beschreiben, annehmen, sich aber von Kernen durch die starke Farbung besonders der peripheren Partie unterscheiden lassen." He then states that he agrees with Ciamician and Weisman as to their origin and that they do not arise directly from nuclei as Tichomiroff had shown, but that the latter author was deceived as to their true nature by their nuclear-like appearance.

Doflein ('97) describes in considerable detail the origin of the pseudo-cells. He cites the views of several of the authors named above, and then shows that in *Tubularia larynx* these structures are the metamorphosed nuclei of the cells which have fused with the egg and whose protoplasm has been added to it. These nuclei in the earliest stage of their metamorphosis differ from the nuclei of the primary germ cells only in that they lie in vacuoles. Their further metamorphosis consists in the disappearance of the vacuoles within the nucleolus, the thickening of the peripheral zone of chromatin matter, and the gradual disappearance of the clear non-staining area about the nucleolus. The nucleolus then enlarges considerably and the change is complete. By the use of a methyl-green-eosin stain he shows that a chemical change takes place in the nuclear substances during the meta-

morphosis. He observed that several of these persistent nuclei are often swept into the same vacuole by streaming movements of the protoplasm. He describes cases of the amitotic division of these nuclei as being of frequent occurrence within the egg.

Allen ('00) describes the changes undergone by the engulfed nuclei in the growing egg of *Tubularia crocea*. Many nuclei were found within the cytoplasm of the egg which showed no difference from the nuclei of the cells of the germinal layer.

These changes consisted in the assembling of the chromatin filaments into a varying number of small spheres just within the nuclear membrane, the disappearance of the threads supporting the nucleolus and a chemic change in the character of the ground substance supporting the chromatin, such that it becomes reactive to staining agents. The structure of the nucleus becomes less distinct. A description is also given of amitotic divisions of these nuclei within the cytoplasm of the egg. Her results agree largely with those of Doflein ('97).

Tannreuther ('08) observes that some of the nuclei of the interstitial cells surrounding the egg are taken up by it and transformed into pseudo-cells. The transformation is accomplished by the granulation of the chromatin; its arrangment in a band around the inner border of the nucleus, and the imbedding of the nucleoli within this band thus presenting the "appearance of a hollow sphere with its wall thicked on one side."

Downing ('08) believes them to consist of lecithin which at first is diffuse in the egg but later becomes aggregated to form the pseudo-cells. He notes also that the nuclei of the interstitial cells become filled with it, meantime enlarging considerably. These nuclei, he says, may also eventually give rise to the pseudo-cells.

It is evident from the above that two views have prevailed as to the origin of the pseudo-cell. The one, that held by the earlier writers principally, that they were accumulations or growths within the cytoplasm of the egg; the other, that they are the persistent and metamorphosed nuclei of the cells which fuse or coalesce with the egg in the process of its growth.

My studies have convinced me that they may be due to both of these processes, but that in the *Hydra* at least, these darkly

staining bodies within the cytoplasm of the egg are not alone nuclei, but also nucleoli, or whole cells, and in some cases, the equivalent of two or even three cells, in all of which a certain characteristic change has taken place.

An examination of Fig. 11, Pl. II., and Fig. 14, Pl. III., and Fig. 17, Pl. III., which are camera drawings of a portion of an egg in which these bodies are very darkly stained, will show that there are three or four different sizes; there is usually a darkly staining hemisphere while the other shades off almost imperceptibly into the surrounding cytoplasm, or at most has but a very thin rim of staining material; that some appear as a mere shell of stained matter with a non-staining interior; that a very few are exceedingly large when compared with the rest. All of these different kinds are not sharply differentiated but shade into each other as would naturally be expected from the consideration of the processes next to be described.

The account of the origin of these bodies, which are wrongly called cells, will be given under three headings: (1) Those derived from whole nourishing cells, (2) those derived from the nuclei of the nourishing cells, and (3) those derived from the nucleoli of the nourishing cells.

Pseudo-cells Derived from Whole Cells.

As previously indicated, the egg grows by two processes, one of which has already been described. The other is by the appropriation of the nourishing cells sometimes before any visible change either in size or appearance has taken place. This process is in some cases similar to those which take place when an amœba engulfs food particles. The cytoplasm of the egg gradually surrounds one of the cells and it is finally taken bodily into the egg. Cases of whole cells thus taken into the egg cytoplasm are not rare. Such a one is shown in Fig. 3, Pl. I. The cell wall is perfectly distinct and the cell contents differ little in appearance from those of one of the cells outside of the egg. The irregular, dark shading is a peripheral thickening of the protoplasm, as is evidenced by focusing upon different regions of the cell.

The fact that cells are frequently thus bodily engulfed lends considerable weight to the amœboid theory of the growth of the

egg. On the other hand, the process of adding cells in which an enlargement and regressive change has taken place has no similarity to amœbic activities. It appears, then, that the egg grows not only by processes comparable to amœbic activities, but also by those which have no possible similarity to them.

In all cases examined, the egg, just before breaking through the ectoderm, had appropriated all of the nourishing cells between it and the layer of large ectoderm cells (Fig. 14, Pl. III.). At either side of the egg mass a considerable amount of unused material may still remain after the egg has broken through the ectoderm but it is probable that it is used by another egg or, if conditions do not favor such a process, may be resorbed.

In many of the preparations the nuclei of the nourishing cells were possessed of several nucleolar bodies in addition to the nucleolus. These were sometimes darkly staining, but more often of a yellowish color. Often some were closely applied to the nucleolus and others to the nuclear membrane. Extremely irregular masses are often thus formed. Such nuclei are shown in Fig. 20, Pl. III. More will be said about them below.

When brought into the egg, and, as above noted, occasionally outside of it, a change is noticeable in the appearance and staining of the cells. In the case of whole cells these changes are as follows: The nucleus approaches the periphery of the cell; the nucleoli become peripherally arranged within the nuclear membrane and often appear to be pushing outward as the membrane is frequently extremely irregular and sometimes broken though this phenomenon is not always to be observed (Fig. 21, *a*, Pl. III.). The chromatin filaments are visible for a time but are much less distinct than in the normal nucleus. Whether or not the chromatin collects in the form of small spherules which resemble the nucleoli, I have been unable to determine. In some cases it seems to do so. Allen ('00) describes such a process for *Tubularia crocea*.

In the next stage the nucleolar bodies appear to coalesce in part, as their identity can no longer be recognized. The resulting mass is irregular in outline and very darkly staining. At the same time the region about it begins to react slightly to the stain (Fig. 21, *b*, Pl. III.). The chromatin filaments are now entirely

indistinguishable. The third stage resembles the one just described, but the hemisphere in which the nucleolar mass lies is also very darkly staining, so much so that the mass within it is often entirely obscured. By sufficiently destaining, however, one can distinguish this mass in almost any pseudo-cell of this sort. The nuclear membrane is broken early in the changes above described and is apparently entirely lost. The last stage is shown in Fig. 21, *c*, Pl. III. The drawings are taken from typical cases and many variations are to be found, but all are easily explained according to the above account.

Other pseudo-cells of much greater size are frequently found, and their origin can be explained in much the same way. Outside of the egg one sometimes finds that some of the cells have more than one nucleus, rarely as many as four or five. This fact has already been mentioned, and in connection with it a statement as to their probable origin, viz., by the union of two or more of the primitive ova rather than by the divisions of the nucleus of one of them. This conclusion was the result of the observation that no evidences of divisions in any of the nuclei of these cells were found, though such multi-nucleated cells were not uncommon. If divisions do occur the evidences are for their taking place amitotically, though I am inclined to believe that such divisions do not take place. The metamorphosis of these large cells into pseudo-cells takes place in the same way as that of the single-nucleated cell.

Occasionally a pseudo-cell of comparatively enormous size is found, such as that shown in Fig. 11, Pl. II. These are evidently derived from still larger multi-nucleated masses, which are sometimes found outside the egg. Fig. 19, Pl. II., shows such a cell with four nuclei and evidences of a fifth. I have found but this one instance, however, and it is therefore very unusual. Brauer ('91*a*) has shown a large pseudo-cell in his Fig. 4, Pl. X., and they are therefore not an unusual structure. The changes involved in their metamorphosis are probably the same as those already described. No intermediate stages were found and therefore no figures can be given.

In no cases have I found convincing evidences of the amitotic nuclear divisions of these bodies such as have been described by Ciamician ('79), Doflein ('97), Allen ('00) and Tannreuther ('08).

An explanation of the phenomena here described, though not easy to find, appears to be as follows: The nucleolar bodies when they appear within the nucleus in such large numbers are in small part products of the metabolic changes within the nucleus but in greater part are doubtless certain liquids which have osmotically penetrated the nuclear membrane and been coagulated by the killing and hardening fluids. These substances may serve as food for the nuclear activities and, in their later history, as stores of energy for the embryo. The ultimate disposition of these bodies in the cell would support this view. No attempt was made to determine their chemical nature by staining methods as the material required was lacking. Similar nucleolar bodies have been described by Montgomery ('98) in the case of certain nemertean worms.[1] By staining methods he seems to have shown quite conclusively that they are liquids taken in through the nuclear membrane and are of the same nature as the yolk balls which occur very abundantly within the cytoplasm. Such yolk masses are growths or accumulations and as such are different than the pseudo-cells which are found in the egg under discussion.

These nucleolar bodies usually appear in the first stage of the metamorphosis of the cell or nucleus. As indicated above, it is not probable that the chromatin collects in small spherules to add to the number. The nuclear membrane in this stage is frequently broken and very irregular. The second stage is due to a partial fusion or flowing together of these bodies. The rupture of the nuclear membrane results in the liberation of the nuclear liquids, in the case of the metamorphosis of a whole cell, which are apparently separated by some chemical factor so that the chromatin substance becomes spread out in the periphery of the hemisphere in which the nucleus lies, thus accounting for the darkly staining properties of this portion of the structure. At the same time the cytoplasm itself becomes chemically changed, for it reacts slightly to a chromatin stain. The nuclear sap does not appear to take any differential stain but is disseminated through the cytoplasm of the cell.

That this is the true explanation is evidenced by cross-sections

[1] Principally in *Stichostemma eilhardi*, p. 437; *Zygonemertes virescens*, p. 483; *Tetrastemma elegans*, p. 431.

of the pseudo-cell (Fig. 16, *a* and *b*, Pl. III.). Here one sees a thin rim of darkly staining material which is usually more prominent in one hemisphere though occasionally a continuous one is found (Fig. 16, *b*). Within this rim are the nucleolar bodies often forming a considerable mass or, as in the figure, spread out singly with their edges closely applied to each other. The interior of the structure takes a plasma stain but slightly and is very homogeneous in character. Fig. 16, *c*, Pl. III., is a drawing of a pseudo-cell in which some of the nucleolar bodies were in the pole opposite to that in which the darkly staining mass was found. They are evidently of a different nature than the others as the stain is apparently largely a plasma one.

The cause for the changes above outlined may possibly be due, as previously noted, to a substance secreted by the cytoplasm of the egg. This evidently causes a separation of the nuclear elements and they become arranged as above described. Further study on the processes which take place in the absorption of these bodies may serve to make clear the advantage, if any, which results from the arrangement of the material after the fashion above described. Sometimes the pseudo-cell lies in a conspicuous vacuole and at others none can be distinguished. The metamorphic changes are not entirely comparable to digestive processes for they cease for a time with the peripheral disposition of the darkly staining elements. The nuclein, which is highly resistant to digestive ferments, may serve to protect the contents of the structure from further changes.

2. Pseudo-cells Derived from Nuclei.

There are a considerable number of pseudo-cells which are derived from the nuclei of the nourishing cells. In such cases, after being taken into or fusing with it, the cytoplasm seems to blend with that of the egg and then changes occur in the remaining nuclei which are similar to those described as taking place in the nucleus of the whole cells which are transformed into pseudo-cells. The nucleolar bodies do not cause the rupture of the nuclear membrane, but there is an evident separation of the nuclear elements, as the peripheral darkly staining rim clearly shows (Fig. 17, Pl. III.). This process is comparable

to that described by Doflein ('97) and Allen ('00) for the pseudo-cells of *Tubularia.* I have found no cases of division of these bodies.

3. Pseudo-cells Derived from Nucleoli.

It has already been shown that the nucleolus of the nourishing cell sometimes migrates out of the nucleus, leaving a broken and partially destroyed nuclear membrane. After such a cell becomes a part of the egg the nucleolus may persist and form one of the minute bodies so plentiful in the egg. Fig. 6, Pl. I., shows the nucleolus migrating out of a nucleus before the union of the cell with the egg.

Nucleoli and other bodies within the nucleus are also set free in the egg cytoplasm by another process. Along the periphery of the egg one often finds examples of the apparent fusion of the cytoplasm of a cell with that of the egg followed by the enlargement of the nucleus in a manner slightly different from that described in the processes concerned with the transformation of whole cells into pseudo-cells. This condition was most common in those preparations which showed the food spherules in the nourishing cells. The outline of the nuclear membrane becomes irregular, often presenting the appearance of the nucleoli as being under an influence forcing them outward through the membrane. The outline of the nucleus may become twice as large as that of the normal. Fig. 20, *a* and *b*, Pl. III., are drawings of such nuclei but are by no means as irregular as many which were found. Whether these peculiar conditions ever result in the formation of a characteristic pseudo-cell or not, I have been unable to determine. That they do not seems highly probable. Their fate seems to be in the setting free of the nucleoli by the breaking or absorption of the nuclear membrane. Conditions which justify this conclusion are shown in Fig. 15, Pl. III., in which a nest of nucleoli are shown. Their appearance and arrangement indicate that the nuclear membrane had just disappeared as no trace of it could be seen. The minute bodies differed slightly in their staining reactions as indicated in the drawing. Probably, too, the metaplasmic bodies previously described as occurring in the cytoplasm of the egg persist and

swell the number of the minute spherules so plentiful in some preparations.

From the above accounts of the origin of the pseudo-cells it is easy to see how, ranging as they do from exceedingly small to very large ones, the conclusion might very naturally be reached that they are products of the cytoplasm of the egg and grow within it, undergoing certain changes in the process. Possibly the metaplasmic bodies which have been described are derived from the cytoplasm. It is not improbable, then, that some of the darkly staining bodies, are, as Kleinenberg and others concluded, accumulations or growths within the egg, but the majority, so large that the process just mentioned may almost be disregarded, are the remains or parts of the nourishing cells which are taken into the egg, and, under certain influences undergo changes resulting in a partial breaking down, and a characteristic disposition of the elements composing them. These changes are not the same in all cœlenterate eggs in which pseudo-cells occur. The hydroid eggs which I have examined (*Tubularia*) showed no minute bodies at all. The pseudo-cells appeared to be derived entirely from the nuclei of the nourishing cells. Possibly the process here described may be found to occur only in periods of excessive sexual activity when food is very abundant.

Nematocysts within the Egg.

In the early part of the paper it was noted that nematocysts may become imbedded in the egg. In several preparations, particularly in the region near the supporting layer, there were found oval bodies, apparently nematocysts, which were completely covered with minute spherical granules. In some few cases a suggestion of the filament within the nematocyst could be seen. The shape and position of these bodies led to the conclusion that they were nematocysts which were being absorbed or digested. The nature of the granules covering their surface is an open question. They may possibly have been digestive granules of some sort.

History of the Nucleus.

The early history of the nucleus is difficult to follow. As previously narrated, the egg begins its growth by the coalescence

of several cells whose cytoplasm unites to form a common mass and some of whose nuclei undergo certain degenerative changes while others apparently remain unchanged within the resulting multinucleate structure. It has also been suggested that all but one of these nuclei are transformed into pseudo-cells while that one becomes the functioning nucleus of the egg. In the early part of these studies it was thought that possibly all of the nuclei became functionless while the functioning nucleus was reformed *de novo*, from the nuclear material set free in the cytoplasm. The examination of a very large number of sections failed to furnish any growing egg without an apparently functioning nucleus. It seems therefore that from the beginning of the growth of the egg one nucleus retains its sovereignty, though it is impossible to maintain that for a time the others do not perform some of the nuclear functions. It is difficult, in examining the egg when in this multinucleate stage, to determine which is the functioning, or to be, the functioning nucleus. As soon, however, as it becomes distinctly recognizable, it has an appearance much as the one shown in Fig. 7, Pl. I. Both the nucleoplasm and the cytoplasm become more granular, but very shortly after, the nucleoplasm appears to be of an homogeneous character such as is shown in Fig. 8, Pl. I. At the same time the nucleoli increase rapidly in number, there being always one large one eccentrically placed within the nucleus. The staining reactions of the smaller nucleolar bodies indicate that they are not all of the same chemical composition. The nucleus is frequently found to be elliptic in section with its long axis at right angles to the supporting layer. The growth of the egg is attended by a corresponding increase in the size of the nucleus; the number of nucleoli increase greatly, while the nucleus, in the meantime, approaches the outer portion of the egg. In some cases there have been counted as many as eighty to ninety nucleolar bodies in a single nucleus. Vacuoles frequently appear in the larger ones.

The darkly staining area noted by Brauer ('91*a*) as occurring near the large nucleolus has been observed many times. It is shown in Fig. 9, Pl. II., and thus highly magnified, is, as he correctly observed, due to an aggregation of exceedingly small bodies, possibly of the same nature as the nucleoli.

There is a definite nuclear membrane closely applied to the cytoplasm.

In some cases a single cross-section has shown three eggs almost fully grown. Their separate masses in such cases are not separated by an egg membrane of any sort, but flow into each other at their boundaries, which are indicated by a lesser thickening of the egg mass. In such cases it appears that there are three distinct areas in which the growth of an egg has begun in the manner previously described. Tannreuther ('08) has made the observation that after the coalescence of the large ova, two or more of the nuclei persist and each becomes a functioning nucleus, and each, presumably, appropriating a portion of the original mass making up the cytoplasmic portion of the growing egg or eggs. My observations have given no evidences of such a condition or process, but indicate rather that egg development begins in two or more points or areas, and growth continues, until, by the total appropriation of the nourishing cells, their masses become continuous.

By the time the egg has attained its growth the nucleus has migrated to the periphery of the egg where it lies in an area free from pseudo-cells, and just beneath the ectoderm. The membrane becomes very indistinct, while a slight shrinkage away from the cytoplasm is noticeable (Fig. 14, Pl. II.). This condition becomes more pronounced for the outline of the nucleus becomes very irregular and almost indistinguishable. This condition, as Brauer ('91*a*) has stated, precedes the formation of the first maturation spindle.

Maturation.

These processes have been described by Brauer ('91*a*) and Tannreuther ('08) for *Hydra* sp.?, and my own observations have added little to the account as given by them. The former writer states that the number of chromosomes is probably twelve or fourteen. Their shape and size make an accurate determination difficult, but I am inclined to consider that the number is as great as sixteen. The polar bodies are easily found in mature eggs, lying just beneath the ectoderm, and may remain attached to the egg by a cytoplasmic strand even after exposed to the water.

Extrusion of the Egg.

As previously noted, the egg begins by a process of coalescence, which may take place in more than one place, so that there may be three or four eggs growing on the same animal at the same time. In such cases the growing eggs form a thick band entirely surrounding the animal, and, in some cases, almost all of the upper third of the body. In other cases but one egg develops, when it appears as a well pronounced dome-shaped elevation, from which, before maturity, pseudopodial processes radiate outward, and may encircle over a half of the circumference of the body. In either case the completion of the process of appropriation of the nourishing cells is marked by the disappearance of the pseudopodial processes, and it then becomes more regular in outline, and more pronouncedly dome-shaped (Fig. 28, Pl. IV.). The ectoderm is then ruptured and quickly withdraws over the egg giving rise then to the bowl-shaped depression, or, if the extent of the area occupied by the egg or eggs makes this process impossible, the ectoderm is ruptured and the egg mass flows out through the opening gradually taking on the spherical shape and the shrunken ectoderm again forming the bowl-shaped depression in which the egg is found always to lie. The whole process rarely occupies more than two minutes and is sometimes accomplished even more quickly. The egg is attached to the body by delicate filaments continuous with the egg membrane.

Fertilization.

The process of fertilization has been described by Brauer ('91*a*) and to his account little can be added. Unless the process is effected within a few hours after the extrusion of the egg, it becomes vacuolated and increases greatly in size, and finally goes to pieces. Numerous eggs were observed which, thus enlarged, gave off a part of themselves after the fashion of budding yeast. These parts thus set free were sometimes as much as one third the volume of the egg. Some eggs were found to be infested by a protozoön which fed upon the egg material. The interior of the egg in such cases contained hundreds of them. Apparently vigorous eggs were not found to be so attacked, but those in which degeneration was going on were frequently infested. It is

not impossible, however, that the healthy and vigorous egg may be sometimes thus infested.

Egg Membrane.

For some time before breaking through the ectoderm the sections show an egg membrane of varying thickness to be present. This is sometimes difficult to find until shortly before the emergence of the egg. When the egg becomes surrounded by water the membrane is invisible owing to its transparency, but quickly becomes evident upon the application of killing fluids.

Cleavage.

The first cleavage is total and equal. It begins at the distal pole of the egg and in a plane usually at right angles to the long axis of the body. Papillæ are well developed at the point where the cleavage begins and are easily seen on either side of the cleavage furrow when very young (Fig. 23, *a*, Pl. III.). This agrees with Kleinenberg's observations on the cleavage of *H. viridis*. The furrow deepens and its edges close over as it advances, its position being shown by an opening extending completely through the egg (Fig. 23, *b* and *c*, Pl. III.). The first cleavage is accomplished in about thirty minutes.

The second cleavage takes place in a plane at right angles to that of the first and is also total and equal. The papillary processes are also to be seen in the cleavage furrow. The four resulting blastomeres are of nearly equal size (Fig. 23, *d*, Pl. III.). The third cleavage takes place in a plane at right angles to the other two or parallel with the long axis of the body. In this case the cleavage furrow is much less distinct and is formed more slowly in some of the blastomeres than in others, with the result that from this point onward the blastomeres vary in size and give rise to an extremely irregular mass of cells (Fig. 23, *e*, *f*, *g* and *h*, Pl. III.). In the third and even later cleavages papillary prominences are frequently to be seen at the beginning of the cleavage furrow. These conditions greatly resemble those described by Kleinenberg for *H. viridis*. The blastomeres vary considerably in size, those at the proximal pole usually being of somewhat greater dimensions. At the conclusion of the third

cleavage the cleavage cavity is established (Fig. 24, Pl. IV.). After the third, the cleavages take place in such an irregular fashion as to make it impossible to follow them. As a result, however, of further divisions, there is formed a hollow mass of cells which in some cases vary but little in size, while other sections show a much greater variation (Figs. 26 and 27, Pl. IV.). This increases in size by the continued tangential division of the cells until a very large blastula is formed (Fig. 27, Pl. IV.). The inner ends of the cells contain the larger portion of the pseudo-cells while the nuclëi are located near the outer part.

Embryonic Layers.

The cells of the blastula above described are nearly of the same size, though some project into the cleavage cavity farther than others. Division of these longer cells occurs and the entoderm is thus formed by a process of delamination. With these daughter cells are carried the larger part of the pseudo-cells, so that when the process of entoderm formation is finished the ectoderm cells are comparatively free from them. By the continued division of the primitive ectodermal cells in this fashion the cleavage cavity becomes almost entirely obliterated. The entoderm cells also divide and the solid mass of cells is formed. That the ectoderm cells may themselves be crowded into the cleavage cavity as observed by Brauer ('91*a*) found no confirmation in the preparations examined. Radial divisions were the most numerous with few tangential divisions.

No uniformity in the size of the eggs produced was observed for the amount of material available for the growth of the egg determines in large part its ultimate size.

The completion of the formation of the entoderm marks the beginning of the processes resulting in the embryonic membranes, which, as in other species of *Hydra,* consist of an outer chitinous and densely spiculate shell and a very thin inner membrane just beneath it. The former takes its origin as follows : The ectodermal cells constituting the outer layer of the embryo send out slender processes as shown in Fig. 13, Pl. II., and Fig. 29, Pl. IV. These are most frequently formed in groups of two or more and are remarkably large when compared with the size of the

whole cell. Rarely more than one is produced by one cell so that the several processes constituting a group are formed from contiguous cells. The ends of the process show a marked curve away from the axis of the group. The growth outward of these processes carries with them at their extremities the egg membrane which remains a noticeable part of the embryo even at the time it is set free from the mother. The ectoderm cells at this time are very long and appear greatly distorted as a result of the great change in form. The outer part of the ectoderm cells then gives rise to a thin chitinous envelope as shown in Fig. 12, Pl. II. The cytoplasm within the envelope and near the extremities of the projections becomes very much more granular than the remaining portion, and takes a darker stain as a result. The chitinous covering continues to thicken, and the protoplasmic contents of the processes apparently give rise to the chitinous material of which the spicules are composed, whether by a transformation of the protoplasm into the chitin or by a process of secretion of the material of which it is composed, I am unable to state. The appearance of the protoplasmic granules just within the chitinous covering of the processes would indicate a deposition of material at that point. As this chitinous envelope becomes thicker, and of course harder, it gives rise to a very effective protective covering. From the above account it is evident that the covering takes its origin from the outer part of the ectoderm cells ; the basal portion containing the nuclei and a few pseudo-cells remains apparently unchanged. Beneath the shell there is now a layer of cells of somewhat smaller dimensions but otherwise differing little from their condition before the formation of chitin. From the outer part of these cells there is then secreted the thin inner membrane, and the process is thus completed.

This account agrees in general with that given by Brauer (91*a*) for *Hydra* sp. ?. After the completion of the process of shell formation the embryo remains attached to the mother by means of two or three tough strands which are also somewhat chitinous. They are apparently identical with those holding the egg after its emergence from the ectoderm. In the meantime the shell becomes harder and sections show its characteristic laminate structure, that is, the delicate lines which give it an appearance

of being so constituted. Usually within two days the embryo falls from the parent and its point of attachment is then marked only by the bowl-shaped portion of the shrunken ectoderm by which the egg was originally covered.

No study was made of the processes concerned with the further development of the embryo and of the cytological changes involved in the transformation of the embryonic layers into the definitive ectoderm and entoderm, nor of the further changes in the pseudo-cells. That marked changes must take place becomes evident when one considers that in the ectoderm there must be developed muscle and nerve cells, interstitial cells and nematocysts, while the pseudo-cells are to be absorbed and utilized by the entoderm cells. It was found that embryos would develop and give rise to young *Hydra* in about three weeks. This, of course, was at the temperature of the laboratory. At the time of first appearance from the shell they are possessed of four short tentacles, which later increase to six or more. All traces of the pseudo-cells have disappeared by the time of the completion of the definitive ectoderm and entoderm.

General Discussion.

As to the true nature of the growth of cœlenterate eggs, two views have long been prevalent. One, commonly known as the amœboid theory, is so denominated on account of the striking likeness of the growing egg to an amœba — this likeness lying in the amœboid-like movements in its migrations from one tissue into another ; in that it often possesses pseudopodial processes like those of an amœba ; and more remarkable still, frequently engulfs neighboring cells much after the fashion of an amœba taking in food particles. The opposing theory disavows the likeness, maintaining that the egg grows by the dissolution of the cell walls between the egg and the cells contiguous to it, and that this process with its resulting conditions has no likeness to true amœboid activities.

The former view was first suggested by Balfour ('80) and upheld by Weismann ('83), Metchnikoff ('86), Brauer ('91) and conditions apparently supporting it have been described by many more recent writers. Doflein ('97) was the first to publish ob-

servations seriously opposing it. He described the growth of a cœlenterate egg as taking place by the fusion or blending of the egg with its neighboring cells, and pointed out further, that vacuoles do not form about the engulfed cells in the case of those eggs described by the supporters of the theory, but only about their persistent nuclei. He concluded, therefore, that the amœba likeness was a misconception. Ciamician ('79) had previously described the enlargement of an egg by the coalescence of the egg and the cells surrounding it.

The present conception may perhaps best be indicated by quoting from Wilson ('00)[1] who states that the egg cell in cœlenterates may move actively about in the neighboring cells like an amœba and "in such cases (hydroids) the egg may actively feed upon the surrounding cells, taking them bodily into its substance, or fusing with them and assimilating their substance with its own. In such cases (*Tubularia*, *Hydra*) the nuclei of the food cells long persist in the egg cytoplasm forming the so-called 'pseudo-cells' but finally degenerate and are absorbed by the egg."

It does not seem necessary to assume any active amœboid propensities on the part of the egg of *Hydra* to account for the processes concerned in its growth. That the egg in its early history is entirely devoid of any similarities to an amœba is evident from the account given above of its growth at that time. The coalescence of the primitive ova is surely not comparable to any amœbic activity. Nor do the ova migrate from one part of the body to another as in the case of certain hydroids. The growing egg does not, at first, at any rate, feed upon the surrounding cells after the manner of an amœba engulfing food particles. That the surrounding cells become a part of the egg is true, but the process by which the union is accomplished is simply a coalescence of a group of cells to form a larger mass over which a single nucleus comes in time to hold sovereignty. Possibly but one nucleus is functional from the beginning of the process. Possibly one cell is at all times the controlling factor in all of the processes, but this is difficult to prove.

Nor does it seem necessary to assume any active amœboid properties on the part of the egg in order to account for the fact

[1] "The Cell in Development and Inheritance," p. 150.

that cells are taken bodily into it. Indeed, the facts already set forth are not at all comparable to amœboid activities. Some cases were found in which it would appear that the nourishing cells over a limited area had been transformed into pseudo-cells even before entering the egg. Occasionally, isolated cases of such changes were found at a considerable distance from the egg. Again, the protoplasmic mass resulting from the processes above described is under a certain pressure due to the tension of the ectodermal layer by which it is covered, and would therefore tend to flow into intercellular spaces and often simulate active amœbic engulfments by partially surrounding a cell. It seems more probable that the egg by some chemic influence causes a profound change to take place in the cells which surround it. Possibly the regressive changes, including the formation of the pseudo-cells, is due to this cause. The amœboid form of the egg does exist but perhaps due in part, at least, to the causes suggested above. Just before breaking through the ectoderm, in all cases examined, the egg had appropriated all of the nourishing cells between it and the ectoderm. Rarely, there are groups of cells on either side of the egg which have not been appropriated. The rounding off process described for the eggs of cœlenterates is not due entirely to any activity on the part of the egg of *Hydra* to effect such a form, but is the result, in large part, of the consumption of all of the available nourishing cells within the germinal area so that a more or less regular outline results. That undoubted similarities between the growing egg and an amœba do exist cannot be denied, but I am persuaded that these likenesses are frequently too widely applied and lead to misconceptions.

Summary.

1. The egg begins its growth by the coalescence of a group of the primitive ova; this process is frequently attended by a peculiar nuclear degeneration. One of the nuclei from the group becomes the functioning nucleus; the others either disappear in the cytoplasm or are transformed into pseudo-cells.

2. The egg thus beginning its growth continues to increase in size by two processes: (*a*) By the disappearance of the cell wall between it and neighboring cells attended by a regressive

change in the nucleus which later becomes scattered and absorbed in the cytoplasm. (*b*) By a union with neighboring cells which later, either in whole or in part, become transformed into the so-called pseudo-cells. This process may consist in the engulfment of whole cells.

3. The pseudo-cells are metamorphosed whole cells, nuclei or nucleoli, in which certain characteristic changes have been brought about. These changes are doubtless produced by a chemic influence from the egg.

This paper was undertaken at the suggestion of Dr. C. W. Hargitt. I desire to express my sense of obligation to him for his valuable criticisms and helpful suggestions.

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EXPLANATION OF PLATE I.

FIG. 1. Longitudinal section through a portion of the germinal area. The character of the cells is well shown, as also the metaplasmic bodies frequently occurring in these cells. The double character of the nucleoli is noticeable. That they are composed of different material is evident from the difference in staining capacity. A nematocyst (*nem.*) is shown imbedded in the mass of cells. $\times$ 975.

FIG. 2. A group of the primitive ova in which the regressive changes are just started. In the cell marked (*a*) the chromatin is retreating toward the periphery of the nucleus; in that marked (*b*) the spireme is formed. The cells are a little larger than the other primitive ova which are shown in the upper right hand corner. $\times$ 850.

FIG. 3. The distal portion of a growing egg in which the outline of an engulfed cell is clearly shown. The cell-wall appears as a well-defined line. $\times$ 850.

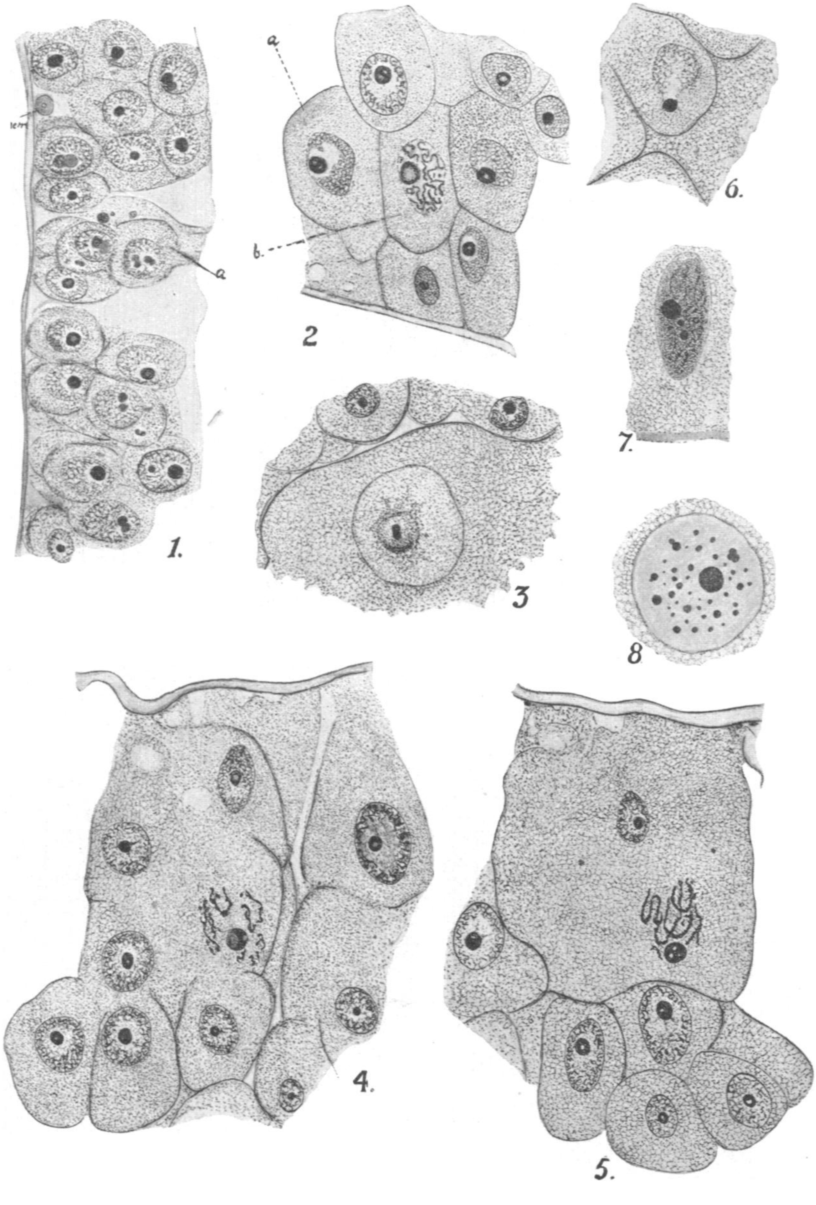
FIG. 4. The fragmentation of the nucleus of one of the cells contributing to the mass of the egg. The cell-wall has in part disappeared, while the coalescence with a neighbor cell has begun. The nuclear membrane has dissolved and the chromatin filaments are being scattered. Vacuoles are conspicuous in the nucleolus. $\times$ 850.

FIG. 5. A condition not quite so far advanced as that in Fig. 4. Several metaplasmic bodies are apparent. In the cells bordering on the egg, the regressive nuclear changes have begun. $\times$ 850.

FIG. 6. A nourishing cell bordering on the egg. The nucleolus has broken through the nuclear membrane. Within, the broken chromatin filaments are plainly visible. $\times$ 650.

FIG. 7. A young nucleus showing the single large nucleolus and several small nucleoli. Chromatin filaments are evident. $\times$ 650.

FIG. 8. An older nucleus in which the nucleoli are very numerous. There are no traces of chromatin filaments whatsoever. $\times$ 650.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Explanation of Plate II.

FIG. 9 A drawing of a part of a growing egg showing the nucleus in the process of shortening from the elongated form. The character of the cells outside of the egg itself is typical. In one of them the regressive changes are well under way, the nuclear membrane having disappeared entirely, and the chromatin filaments set free in the cytoplasm. One of the very large cells, probably resulting from the coalescence of two cells, is likewise shown. Darkly staining lines in chromatin material indicate the probable fate of the nuclear stuff.

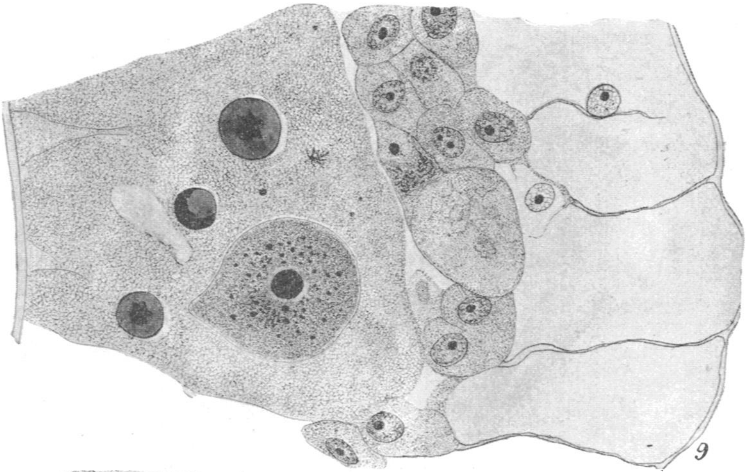
Within the nucleus of the egg are a large number of nucleoli and a single large nucleolus. In the cytoplasm is a tangle of filaments evidently the remnants of a cell whose mass has been added to that of the egg. Several metaplasmic bodies are also visible. $\times$ 650.

FIG. 10. A portion of a growing egg and a group of cells upon its periphery. The very large cell, probably derived from the fusion of two or more, is about to unite with the egg mass. In its nucleus the regressive changes are well advanced. In the small cell outside of this large one, the chromatin has assembled in the spireme, preparatory to union with the large cell. $\times$ 650.

FIG. 11. A part of an egg in which occurred one of the very large pseudo-cells. Three thickened and darkly staining portions of its rim are noticeable. Along its edges, in two places at least, one can see the nucleoli from the cells contributing to the mass. $\times$ 650.

FIG. 12. A portion of the ectodermal layer of a fully segmented egg showing the manner of outgrowth of the cells previous to the formation of the chitinous shell. $\times$ 650.

FIG. 13. The beginning of the shell formation by the development of the chitinous layer outside of the ectodermal processes. $\times$ 650.



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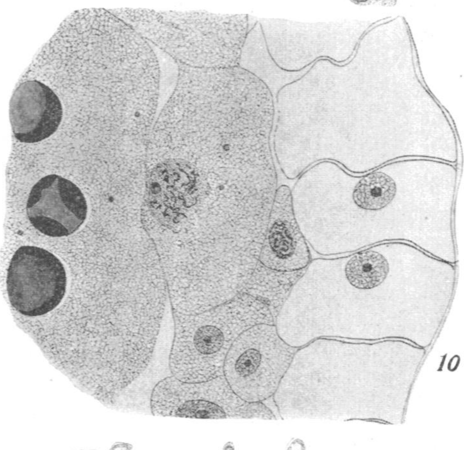

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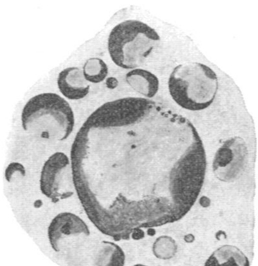

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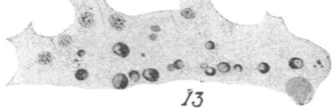

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EXPLANATION OF PLATE III.

FIG. 14. Distal portion of an egg at about the time of its breaking through the ectoderm, and previous to the process of maturation. The nucleus at this time becomes less darkly staining, and irregular in outline. The pseudo-cells are typical in that there are shown the variety of forms and sizes. $\times$ 650.

FIG. 15. A nest of nucleolar bodies which have evidently been derived from a single nucleus by the dissolution of the nuclear membrane. These stain with different degrees of intensity, and in double staining, in different colors, indicating their difference in composition. $\times$ 850.

FIG. 16. *a*, section through a pseudo-cell showing the thin rim of darkly staining material and the nucleoli arranged within it. *b*, another section in which the peripheral disposition of the nucleolar masses is conspicuous. *c*, a pseudo-cell in which the nucleoli are shown in the pole opposite the darkly staining one. In this case they take a distinct plasma stain when double stained. $\times$ 850.

FIG. 17. Cross-section of a pseudo-cell probably derived from a nucleus. The peripheral disposition of the chromatin is apparent in this as in the other cases. $\times$ 850.

FIG. 18. A pseudo-cell which shows its triple origin. Three nuclear masses are apparent as also three separate areas which stain darkly. $\times$ 850.

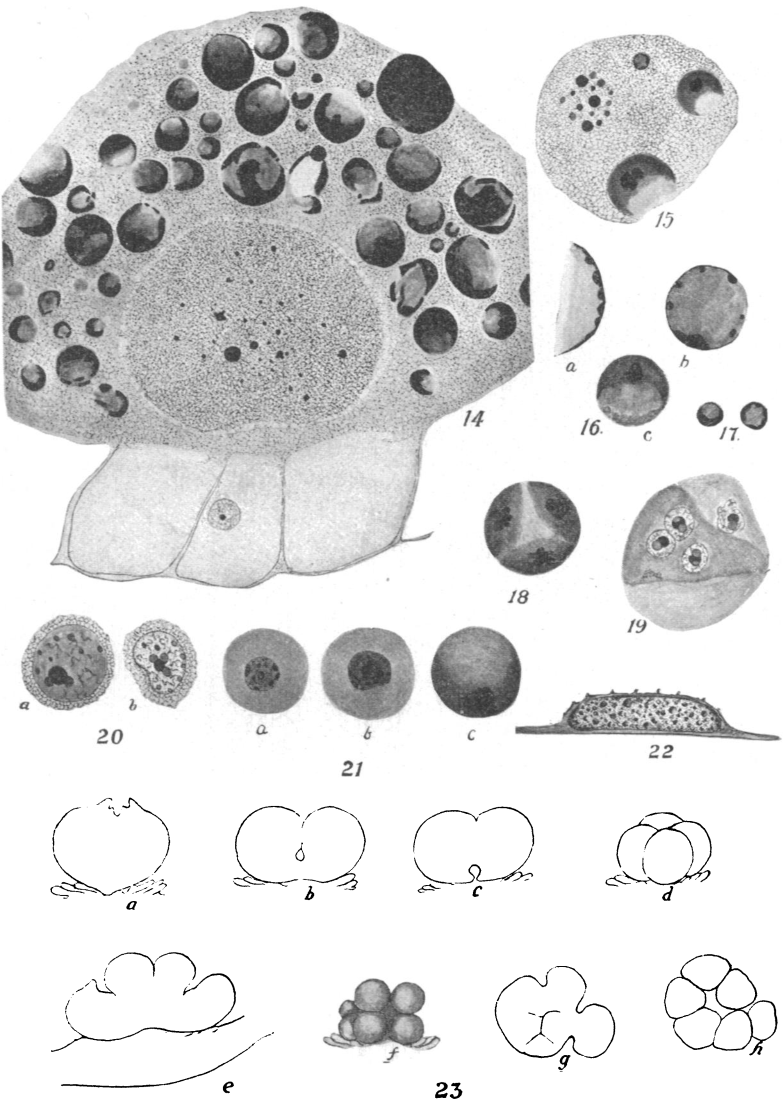
FIG. 19. A very large body found just outside of a growing egg, and in which were shown four well defined nuclei and traces of a fifth. Such conditions were found but once and may have been an artifact. However, the very large size of some of the pseudo-cells indicates that such large masses of coalesced cells frequently occur. $\times$ 850.

FIG. 20. A number of nuclei as found in some of the cells outside of the egg. There were in some a number of nucleolar bodies which were not of true chromatic origin but apparently food masses within the nucleus. They were of a yellowish color. These were not found in all cases, but those sections on which they were found were apparently well fixed and stained. Possibly their presence depended upon certain metabolic processes. $\times$ 850.

FIG. 21. Three stages in the metamorphosis of a whole cell into a pseudo-cell. In *a*, the outline of the nucleus is still evident and traces of the chromatin filaments are also visible. The nucleoli are peripherally arranged within the nucleus. In *b*, the nucleolar bodies have become massed together and the hemisphere of the cell in which they lie is slightly more darkly staining than in the previous figure. In *c*, the transformation is complete. The nucleolar mass is visible, but its containing hemisphere is now very darkly staining, the boundary of the stained being sometimes sharply defined from the unstained portion. $\times$ 650.

FIG. 22. One of the flattened short spiculated eggs ; drawn from a section.

FIG. 23. A series of drawings of a segmenting egg. *a*, *b* and *c*, the beginning, middle and ending, of the first cleavage. *d*, the four-celled stage. Up to this time cleavage is regular, total and equal. *e*, drawing showing the irregularity of the later cleavages. In this case the cleavage lines were not entirely visible *f*, *g* and *h*, later stages, showing more pronounced irregularities.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Explanation of Plate IV.

Fig. 24. Equatorial section through the egg segmented to the eight-celled stage. The cleavage cavity and protoplasmic bridges are well shown.

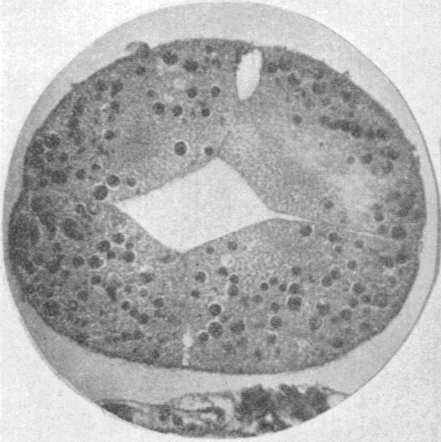
Fig. 25. Similar section through the egg at a little later stage. The cleavage cavity is more definite and a variation in the size of the blastomeres is noticeable.

Fig. 26. Section through the segmented egg at a more advanced stage. In this case the process seems to have been unusually regular. The blastomeres are apparently of nearly uniform size, and regularly arranged.

Fig. 27. Segmentation carried to the completion of the hollow mass of cells just before the beginning of the formation of the primitive entoderm cells. Some of the cells are noticeably larger and longer than others.

Fig. 28. The growth of the egg completed. The first polar body is to be seen just beneath the ectodermal covering. The egg membrane is also visible as a thin slightly shaded area beneath the ectoderm.

Fig. 29. The protoplasmic process from the ectodermal cells about which the spiculate structures are formed. The chitinous envelope is already forming. The dark spot in one of the processes is not a pseudo-cell, but a piece of foreign matter in the preparation.



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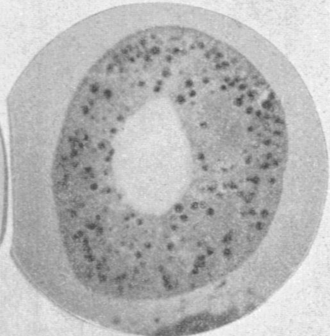

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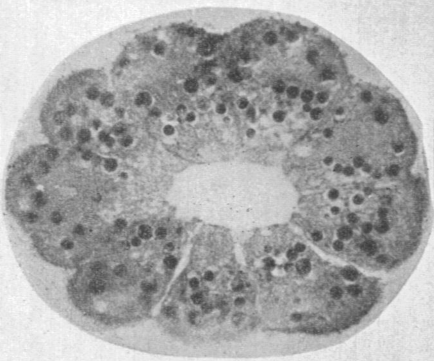

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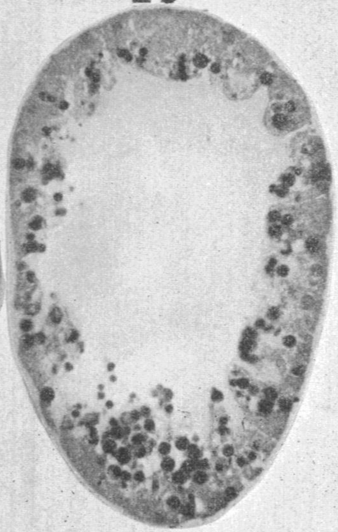

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